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Developmental Neurobehavioral Effects on JP-8 Jet Fuel on Pups from Female Sprague-Dawley Rats Exposed by Oral Gavage

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FOR THE DIRECTOR

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13. ABSTRACT *(Maximum 200 words)*

U.S. military personnel are exposed frequently to the jet fuel JP-8 since it became the standardized military fuel. Limited reproductive studies in rats have been conducted with JP-8. Female rats were dosed with 0, 325, 750 or 1500 mg/kg-day neat JP-8 by gavage for 21 weeks, including 90 days prior to cohabitation, gestation and lactation. Pup body weights from the high dose were significantly decreased ($p<0.05$) compared to controls on postnatal days (PNDs) 4 through 21. No reproductive effects were found in this study including no group differences in testis decent and vaginal patency. Effects of JP-8 exposure were found in results from developmental neurobehavioral testing. Litters were standardized to four male and four female pups at PND 4; all eight pups in a litter were tested for surface righting and negative geotaxis. JP-8 did not affect age of onset for surface righting reflex in pups tested on PND 4. Negative geotaxis abilities, tested on PNDs 5 through 8, developed at the same age for pups in all JP-8 groups; however, all females met the criterion sooner than males. Development of motor coordination related to swimming was tested in one male and one female pup from each litter every other day from PNDs 6 through 20. A dose-related difference in composite scores for swimming abilities was observed on PNDs 8 and 14, indicating a delay in development of coordinated motor movements related to the swimming task. On PND 8, pup scores from all doses were ≥20% lower than control scores. On PND 14, composite swimming scores were 8% lower in the 750 and 1500 mg/kg-day dose groups versus controls. Pups were tested in an M swimming maze on PNDs 70 and 77. JP-8 did not affect the number of trials to criterion on either test date; on PND 77, male pups met the criterion of five errorless trials in fewer attempts than females.

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PREFACE

This study was conducted in 1993 at the Armstrong Laboratory, Occupational and Environmental Health Directorate (AL/OET), currently the Air Force Research Laboratory, Human Effectiveness Directorate, Operational Toxicology Branch (AFRL/HEST). James R. Cooper, Brenda D. Schimmel, Timothy A. Bausman and Susan M. Young were government employees at that time. Results were compiled by Teresa R. Sterner (Operational Technologies Corporation, DAHA 90-96-D-0014, Manager: Dr. Peter Lurker, 1370 N. Fairfield Rd., Suite A, Beavercreek, Ohio 45432) with extensive assistance by the former government technicians: Brenda Schimmel (Operational Technologies Corp.) and Timothy Bausman and Susan Young (ManTech Environmental Technology, ManTech GEOCENTERS Joint Venture, F33615-00-C-6060, Program Manager: Dr. Darol Dodd, P.O. Box 31009, Dayton, Ohio 45437). Dr. Cooper is currently with Children's Hospital Research Foundation (Columbus, Ohio 43205).

The animals used in this study were handled in accordance with the principles in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, DHHS, National Institute of Health Publication #86-23, 1985 and the Animal Welfare Act of 1966, as amended.

The authors would like to acknowledge Chuck Goodyear for performing statistical analyses of the data. Mr. Goodyear is a statistical consultant for AFRL, Human Effectiveness Directorate, Crew Systems Interface Division (AFRL/HEC), Wright-Patterson Air Force Base, Ohio.

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LIST OF ACRONYMS

°C	degrees Celsius
°F	degrees Fahrenheit
ALT	alanine aminotransferase
ANOVA	analysis of variance
ARAS	appetitive reinforcer approach sensitization
AST	aspartate aminotransferase
g	gram
kg	kilogram
LOAEL	lowest observed adverse effect level
m^3	cubic meter
mg	milligram
mL	milliliter
NOAEL	no observed adverse effect level
PND	postnatal day

DEVELOPMENTAL NEUROBEHAVIORAL EFFECTS OF JP-8 JET FUEL ON PUPS FROM FEMALE SPRAGUE-DAWLEY RATS EXPOSED BY ORAL GAVAGE

INTRODUCTION

U.S. military personnel are exposed frequently to vapor, aerosol and liquid forms of the jet fuel JP-8 since it became the standardized military fuel[1]. Human exposure to JP-8 through dermal and inhalation routes has the potential to be harmful. Dermal absorption was not found to be sufficient to cause systemic toxicity under normal occupational conditions; however, dermal exposure can cause skin irritation[2]. Respiratory irritation caused by exhaust has been reported in Japanese Air Force workers[3]. Recent studies have confirmed that the database for JP-8 toxicity is not complete. Neurobehavioral studies are needed to understand the effects of JP-8 on the central nervous systems of humans and animals during development and as adults.

Mattie *et al.* (1991) conducted a subchronic inhalation toxicity test of JP-8 by exposing male and female F-344 rats and C57BL/6 mice to JP-8 vapors at 0, 500 and 1,000 mg/m^3 on a continuous basis for 90 days, followed by recovery until approximately 24 months of age. Evaluation of data revealed limited toxicity and no tumor formation. Effects seen were either not a direct treatment effect of JP-8 or were due to the male rat specific alpha 2-microglobulin protein droplet nephropathy[4]. The nephropathy seen in male rats is not expected to occur in people[5]; therefore, the limited toxicity seen in the JP-8 repeated dose study was not relevant to humans.

The data reported in this technical publication were part of a multi-step investigation into the oral toxicity of JP-8. Clinical and histopathology parameters for the male rats in this study were previously reported by Mattie *et al.*[6] Male Sprague-Dawley rats were dosed with neat JP-8 (0, 750, 1500, 3000 mg/kg) daily by gavage for 90 days. Results revealed a significant dose-dependent decrease in body weights of rats exposed to JP-8. The male rat-specific alpha 2-microglobulin nephropathy was observed by histopathologic examination. A number of significant changes were also seen in blood and urine that were not dose-dependent. Additional treatment-related effects were gastritis and perianal dermatitis. Although there were no histopathological or weight changes in the livers of exposed rats, there were increases in the liver enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase). The elevated enzymes did not increase with increasing dose of JP-8.

A developmental toxicity study indicated that JP-8 is not a teratogen in the rat. Female rats were dosed with 0, 500, 1000, 1500 or 2000 mg/kg neat JP-8 daily by gavage on days 6 through 15 of gestation. Maternal body weights significantly decreased in the 1000, 1500 and 2000 mg/kg-day dose groups while fetal weights decreased in the 1500 and 2000 mg/kg-day groups. Fetal malformations and variations did not differ significantly between control and treatment groups[7].

As part of the aforementioned multi-step investigation, two reproductive studies were performed. JP-8 was shown not to be a reproductive toxicant in rats. In the first study, male rats were given 0, 750, 1500 or 3000 mg/kg neat JP-8 daily by gavage for 70 days prior to mating with naïve females to assess fertility and sperm parameters. After 70 days of dosing, body weights in the 3000 mg/kg group were over 30% lower than control weights. There were

no significant changes for pregnancy rate, gestation length or sperm parameters as compared to control values[8].

In the second reproductive study, general toxicity, fertility and reproductive endpoints were assessed in female rats dosed with neat JP-8 (0, 325, 750 or 1500 mg/kg) daily by gavage for a total of 21 weeks (90-days plus mating with naïve males, gestation and lactation). Results revealed a significant dose-dependent decrease in body weights of the female rats. Significant organ weight ratio increases were seen for the liver:body, liver:brain and kidney:brain weights. Corresponding histopathologic changes and increases in liver enzymes (ALT, AST) were not observed although there was an increase in liver weight. Significant pathological changes were limited to squamous hyperplasia of the stomach and perianal dermatitis. There were no statistically significant changes from control values for gestation length, pregnancy rate and numbers of pups per litter. There was a trend for decreased pup weight with increasing dose from postnatal days 4 through 21 with the 1500 mg/kg pups statistically and biologically significantly lower on these days. Recovery occurred by 90 days. Based on the results of both reproductive studies, the no observed adverse effect level (NOAEL) for JP-8 reproductive and development effects is 750 mg/kg with 1500 mg/kg as a lowest observed adverse effect level (LOAEL) based on decreased pup weights[8].

The study presented in this report is a continuation of the second reproductive study. The pups from the female study above were assessed for potential developmental neurobehavioral deficiencies.

Neurobehavioral effects have been assessed in adult rats following JP-8 vapor inhalation. Changes in behavioral response were observed in two studies where rats were exposed to 0, 500 or 1000 mg/m^3 for 6 hours/day, 5 days a week for 6 weeks. JP-8 inhalation affected performance on very specific tasks and did not cause a generalized deficit. When animals were subjected to different operant tasks with varying levels of complexity, the low and high exposure groups scored the same as control animals on all tests except for the most complex tasks. In these two operant tests, group differences emerged; low dose animals demonstrated better performance than high dose animals while neither group performed differently from controls[9]. In a second study using the same exposure methods, animals were tested in a large battery of neurobehavior tasks. No exposure group differences were found in acoustic startle responses, forelimb grip strength, nociception, social interaction, the forced swim test, spontaneous locomotor activity, passive avoidance or Morris watermaze performance. However, differences were found in a test for behavioral sensitization. The appetitive stimulus approach sensitization (ARAS) measures the time an animal spends proximal to an appetitive stimulus versus a neutral stimulus. Animals exposed to JP-8 spent more time than control animals investigating the appetitive stimulus, suggesting behavioral sensitization and altered neural pathways related to the dopaminergic system[10]. Overall, the data suggest very specific, versus generalized, neurobehavioral effects of JP-8 vapor exposure in adult rats. The purpose of the current study was to examine developmental neurobehavioral endpoints in pups exposed *in utero* and during lactation to JP-8.

METHODS

Female and male Sprague-Dawley rats (Charles River Breeding Labs, Kingston, NY), weighing 180 to 220 g upon receipt, were quarantined for two weeks prior to exposure. Rats were housed in polycarbonate cages with Beta-Chip Hardwood Laboratory Bedding (Northeastern

Products Corp., Warrensburg, NY). Feed (Formula 5008, Ralston Purina, St. Louis, MO) and water were available *ad libitum*. Ambient temperatures were maintained at 21 to 25°C with a light/dark cycle set at 12-hour intervals. Animals were examined daily for clinical signs of toxicity.

Female rats were randomly assigned to four exposure groups. Groups contained a minimum of 35 female rats. The rats were given 0 (control), 325, 750 or 1500 mg/kg JP-8 daily by gavage for 21 weeks (90 days followed by cohabitation, gestation, delivery and lactation). JP-8 was administered by gavage without a vehicle (neat). Volumes to be administered each day were calculated from the female's individual daily body weights and the density of JP-8 (0.81 g/mL). Control animals were dosed with 1.0 mL distilled water under the same conditions as test groups.

Male rats, not exposed to JP-8, were housed 1:1 with treated female rats. Male rats were euthanized after pregnancy was confirmed; dams were euthanized one day after weaning (Day 22 of lactation). Litters were standardized to four male pups and four female pups on postnatal day (PND) 4. Pup weights were recorded on PNDs 1, 4, 14, 21 and 90. All pups were weaned on PND 21. Male pups were checked for descent of testes on PND 21 or 22. Vaginal patency (opening) was verified in female pups on PND 30. All rats were euthanized by carbon dioxide overdose.

Although 94 dams became pregnant in this study, only the 68 litters consisting of exactly 4 male and 4 female pups on PND 14 were used for statistical analysis. The numbers of litters in each dose group were 22, 16, 15 and 15 for the 0, 325, 750 and 1500 mg/kg-day maternal exposure groups, respectively. All pups were weighed on PND 4, prior to litter standardization; they were not weighed again until PND 14. Only litters for which eight pup weights were recorded on PND 14 were used in neurobehavioral evaluations. This allowed comparison of pups from equally sized litters. These were the same litters used to analyze pup body weight trends reported previously[8].

The JP-8 jet fuel was supplied by the U.S. Air Force (AFRL Propulsion Directorate, Wright-Patterson AFB, OH). The fuel met the requirements of Military Specification MIL-T-83133A.

Developmental Neurobehavioral Battery

Surface Righting

All eight pups in the standard litter were tested for surface righting beginning on PND 4. The pups were placed on their backs in the center of a cloth covered metal table. Each pup was allowed 60 seconds to turn over. Any pup that did not succeed on a test day was tested on consecutive following days until success was achieved.

Statistical Analysis: No analysis was required since nearly all pups demonstrated righting on the first test day (see Results section).

Negative Geotaxis

Negative geotaxis abilities were tested in the standard litters starting on PND 5. The test was performed on a 3.5 x 7.75 inches Plexiglas incline (approximately 30° angle from horizontal) covered with 240 grit silicon carbide sand paper. Pups were placed on their feet facing down the incline. Each pup was given 60 seconds to maneuver its head up the incline. Each pup that did not meet the criterion was tested daily until success was achieved.

Statistical Analysis: Mean postnatal days to criterion were used as the dependent variable in a mixed design analysis of variance (ANOVA) with dose as the between factor and sex as the within factor. Litter was considered a random effect and used in all error terms. The accepted level of significance was set at $p \leq 0.05$ for all statistical analyses.

Swimming Development

Development of motor coordination related to swimming was tested in one male and one female pup from each litter. The same pups were tested every other day from PNDs 6 through 20. Pups were placed in a polycarbonate rodent cage partially filled with water at room temperature (68 to 72°F). If pups sank, they were removed immediately. If pups swam, their swimming ability was scored based on the criteria in Table 1. The pups remained in the water for a maximum of 15 seconds per trial.

TABLE 1. CRITERIA FOR SCORING THE DEVELOPMENT OF SWIMMING ABILITIES.

Score	Performance Areas		
	Direction	**Angle of Head**	**Limb Usage**
0	Sank	Nose Submerged	No Paddling
1	Floated	Nose at Surface, Ears below Water Line	Paddling with All Four Legs
2	Swam in Circle	Nose at Surface, Water Line at Ear Level	Paddling with Hind Limbs, Forelimbs Stationary
3	Swam in Straight or Nearly Straight Line		

Note: Swimming development Total Score is the sum of the three performance area scores with zero as the minimum Total Score and seven as the maximum Total Score possible (3 + 2 + 2).

Statistical Analysis: The Total Score was the sum of Direction, Angle of Head and Limb Usage Scores. A mixed design analysis of variance was used with dose as the between factor and sex as the within factor. Litter was considered a random effect for Total Score analyses only. Categorical analysis (Fisher's exact test) better explains differences among the dose groups for Direction, Angle of Head and Limb Usage criteria. The exact test was used to compare the distributions of sex within each dose group and to compare the distributions of dose group (all four dose groups together and pairwise comparisons).

Water Maze

On PND 70, one male and one female pup from each litter (the same pups that completed the swimming development series) were tested in the water maze. A pup was placed at the starting point at the end of the center arm of the M (Figure 1). When the pup swam to the end of the left or right arm, the ramp was placed in the water and the pup was allowed to get out. This arm (for example, the right arm) was designated as the Incorrect Goal. After drying and resting the pup for at least 15 seconds, the ramp was moved to the opposite arm or Correct Goal (e.g., the left arm). The pup was again placed at the starting point and then guided by hand to the Correct Goal. Time trials were started after the pup was rested (≥15 seconds). A stopwatch was used to record the time the pup took to reach the correct goal, with a maximum swimming time of 60 seconds. Pups were rested 15 seconds between trials. Pups completed sufficient trials to have 5 consecutive errorless runs but were allowed to swim for no more than 15 time trials. Errors were counted as the number of completed incorrect turns. Statistical comparisons were performed on the number of trials taken to meet the criterion.

On PND 77, the same pups were tested again to determine long-term memory. Pups were placed in the water at the starting point and expected to swim to the Correct Goal, which was the arm designated as Correct on PND 70 for each individual pup. Scoring criteria were the same as on PND 70.

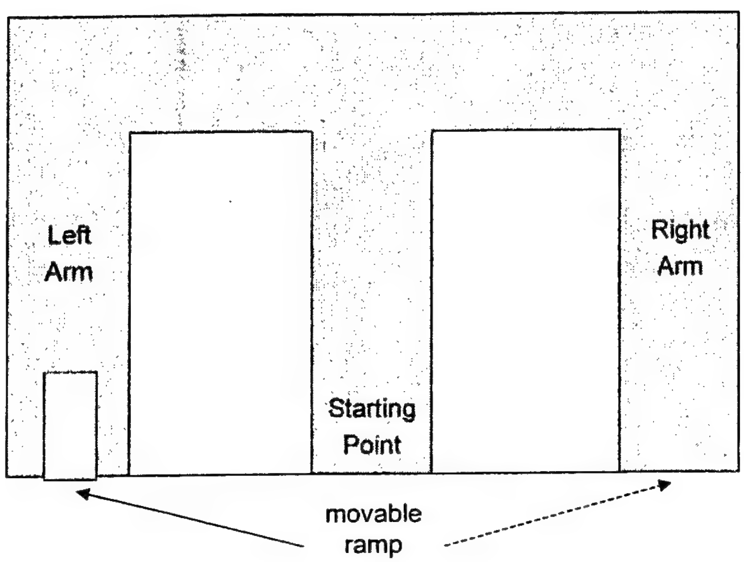

Figure 1. Diagram of water maze showing starting point in center. The ramp can be moved to either the right or left arm.

Statistical Analysis: Two-way ANOVAs were performed separately for PND 70 and PND 77; the dependent variable was the number of trials necessary to reach five consecutive successes. Factors were dose and sex; litters were not considered a factor for these analyses.

RESULTS

Pup body weights from the high dose group were significantly ($p \leq 0.05$) decreased as compared to controls on PNDs 4 through 21, as previously described by Mattie *et al.* (2000)[8]. No group differences were seen in testes descent on PND 21 or 22, or for vaginal patency on PND 30. Table 2 summarizes the results for the neurobehavioral battery.

TABLE 2. SUMMARY RESULTS OF NEUROBEHAVIORAL BATTERY TESTS

Test	Test Days	Dose Effect	Sex Effect
Surface Righting	PND 4 - PND 5	None - Only 10 of the 544 pups (1.8%) required retesting on PND 5	None
Negative Geotaxis	PND 5 - PND 9	No significant difference among the dose groups	Female pups accomplished the task sooner than male pups*
Swimming Development	PND 6	No effect on Total Score; Direction Score significantly lower in 750 & 1500 mg/kg-day groups*	None
Swimming Development	PND 8	Total & Direction Scores significantly lower in 325, 750 & 1500 mg/kg-day groups*	Male pup Total Scores were better than female pup Total Scores; No effect on individual criteria scores*
Swimming Development	PND 10	No significant difference among the dose groups	None
Swimming Development	PND 12	No significant difference among the dose groups	None
Swimming Development	PND 14	Total & Direction Scores significantly lower in 750 & 1500 mg/kg-day groups*	None
Swimming Development	PND 16	No significant difference among the dose groups	None
Swimming Development	PND 18	No significant difference among the dose groups	None
Swimming Development	PND 20	No significant difference among the dose groups	None
Water Maze	PND 70	No significant difference among the dose groups	None
Water Maze	PND 77	No significant difference among the dose groups	Male pups met the criteria in fewer trials than female pups*

* $p \leq 0.05$

Surface Righting

JP-8 did not affect age of onset for surface righting reflex in pups tested on PND 4. Very few pups (10 of the 544 pups or 1.8%) failed to demonstrate the reflex on PND 4 and required retesting on PND 5.

Negative Geotaxis

Each rat pup was tested for negative geotaxis skills beginning on PND 5; all pups successfully performed this test by PND 9. The mean age at which pups met the criterion was determined for males and females of each litter. As shown in Figure 2, there was no significant difference among the dose groups. Negative geotaxis abilities developed at the same age for pups in all JP-8 dose groups. However, there was a significant main effect of sex. The average age of female pups to meet the criterion (mean=6.81 PNDs) was 6% less than the average age of male pups (mean=7.22 PNDs).

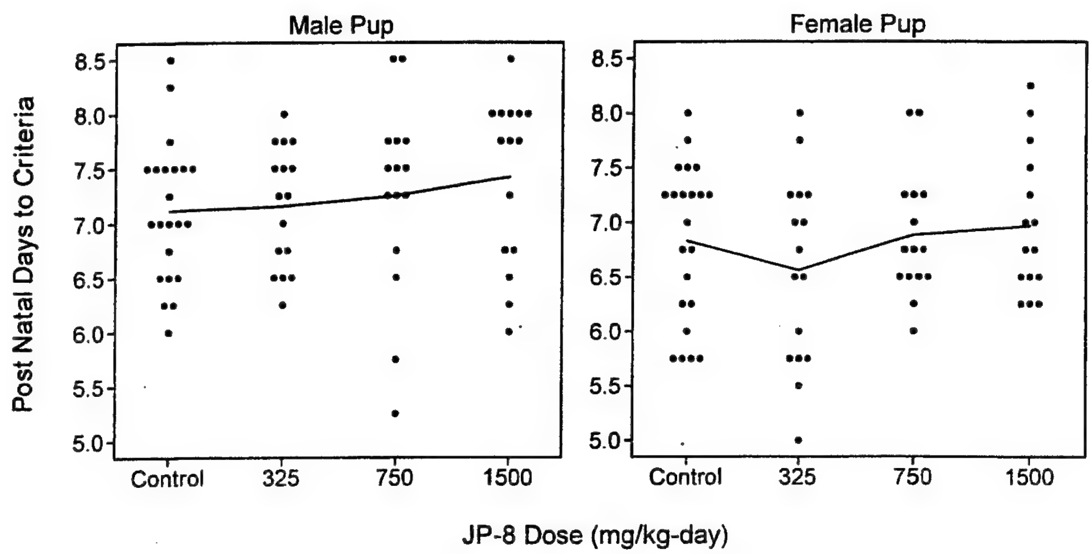

Figure 2. Mean age of four male and four female pups per litter to meet the negative geotaxis criterion. Line segments connect means from each dose group.

Swimming Development

Total Score

Total scores were significantly ($p \leq 0.05$) decreased at all doses on PND 8 and at the two higher doses on PND 14. On PND 8, pup scores from all doses were ≥20% lower than control scores (Figure 3). On PND 14, composite swimming scores were 8% lower in the 750 and 1500 mg/kg-day dose groups versus controls.

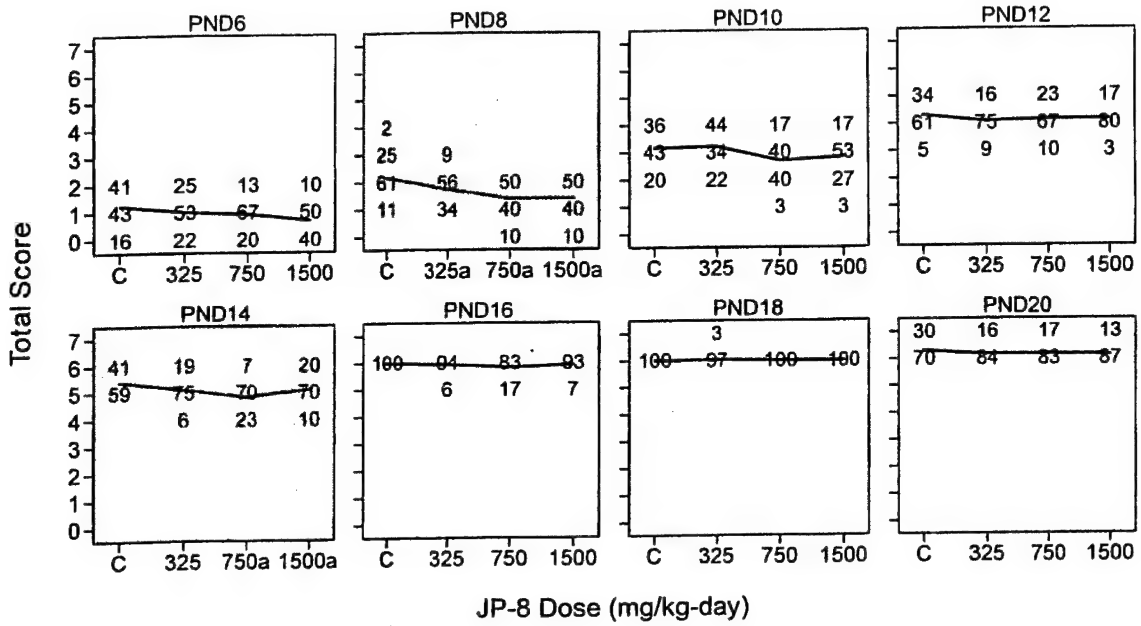

Figure 3. Percent of all pups, irregardless of sex, performing at a particular level of Total Score (sum of Direction, Angle of Head, and Limb Usage criteria) for swimming development (see Table 1 for scoring criteria). Line segments connect means from each dose group (C = control). Comparisons with control (a = p≤0.05) used two-tailed t-tests with pooled error. Test days on which significant differences were found are shaded.

A significant difference was seen in performance of male and female pups at PND 8. Male pups (mean = 1.65) performed 9% better than female pups (mean = 1.82). There were no significant interactions between JP-8 dose group and sex.

Direction, Angle of Head and Limb Usage

There was a significant difference among the dose groups at PNDs 6, 8 and 14 for the Direction criterion (Figure 4) but there were no significant differences among the dose groups for the Angle of Head or Limb Usage criteria at any PND (Figures 5 and 6). There were also no significant differences between the sexes for individual criteria.

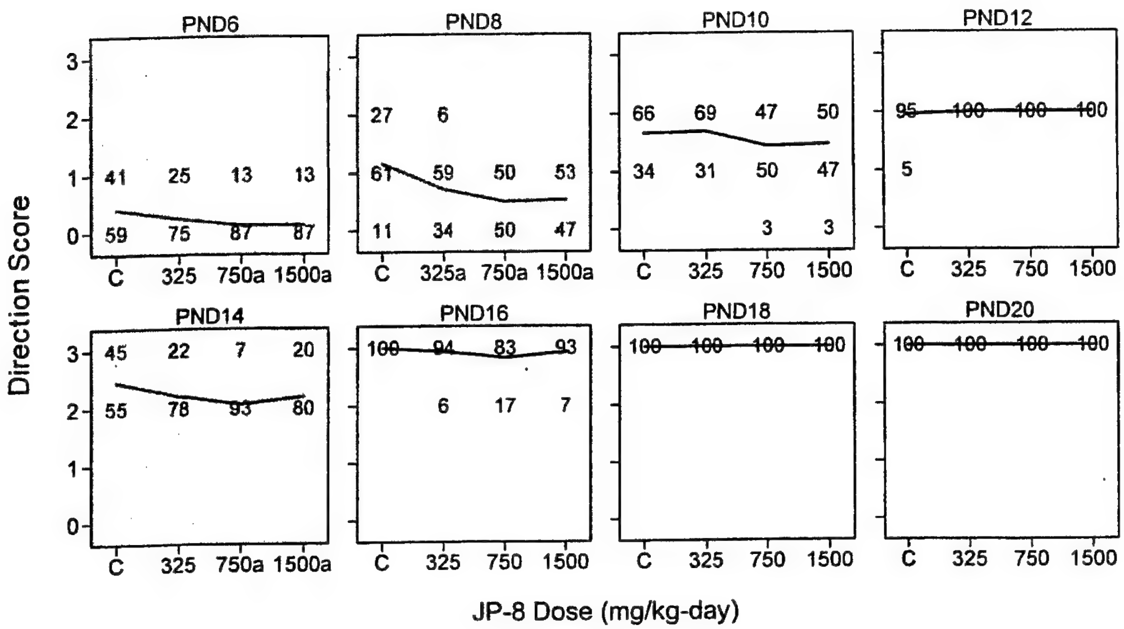

Figure 4. Percent of all pups, irregardless of sex, performing at a particular level of the Direction criterion (see Table 1 for scoring criteria). Line segments connect means from each dose group (C = control). Comparisons with control (a = p≤0.05) were made with Fisher's exact test. Test days on which significant differences were found are shaded.

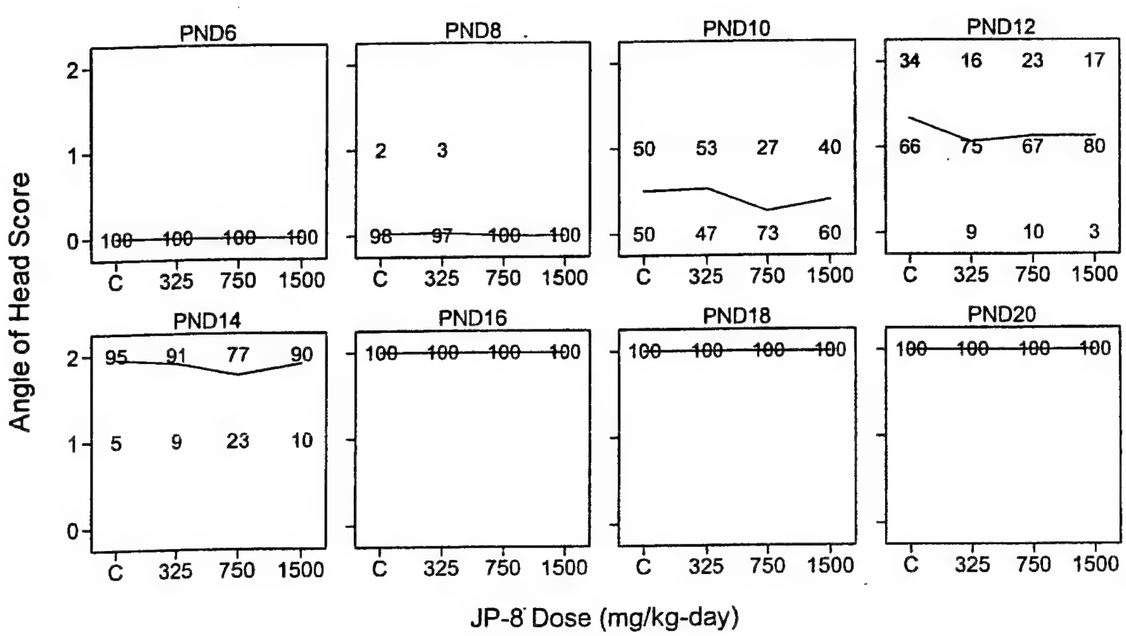

Figure 5. Percent of all pups, irregardless of sex, performing at a particular level of the Angle of Head criterion (see Table 1 for scoring criteria). Line segments connect means from each dose group (C = control).

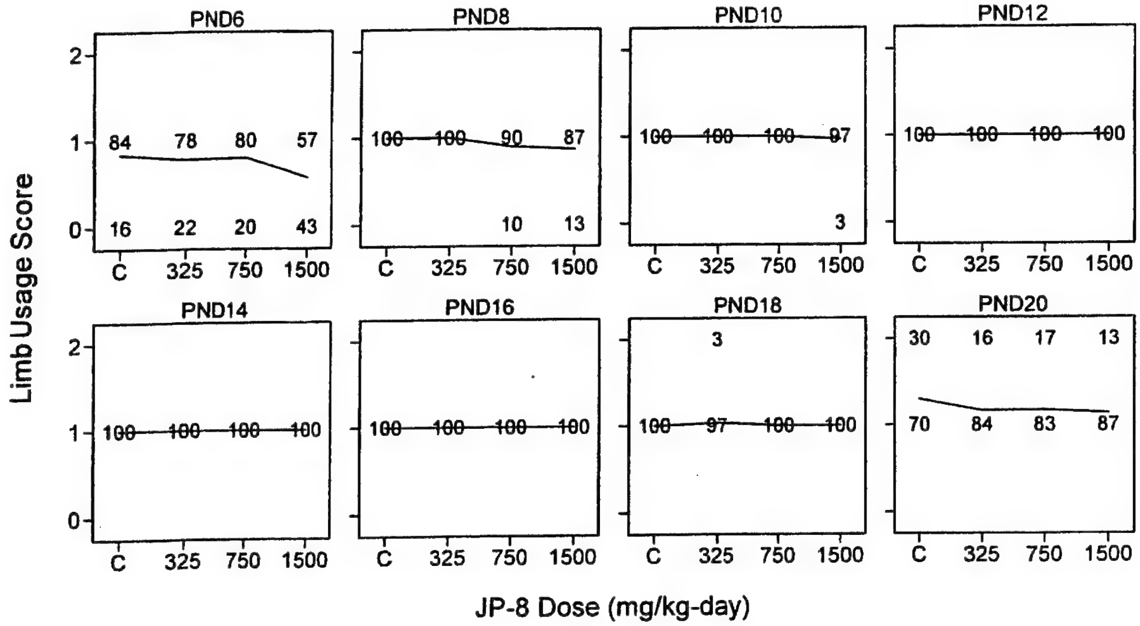

Figure 6. Percent of all pups, irregardless of sex, performing at a particular level of the Limb Usage criterion (see Table 1 for scoring criteria). Line segments connect means from each dose group (C = control).

Water Maze

Two litters from the control group were excluded from the water maze statistical analysis as one of the pups failed to meet the established criterion (i.e., succeed five times in a row by the 15th trial) on PND 70. One litter in the 750 mg/kg maternal dose group was also excluded as the male pup died on PND 69.

As shown in Figures 7 and 8, there was not a significant difference between dose groups at PND 70 or 77. No significant interaction between dose group and sex was found. Although there was no main effect of sex at PND 70, at PND 77 male pups (mean=5.86) met the criterion in fewer trials than female pups (mean=6.40).

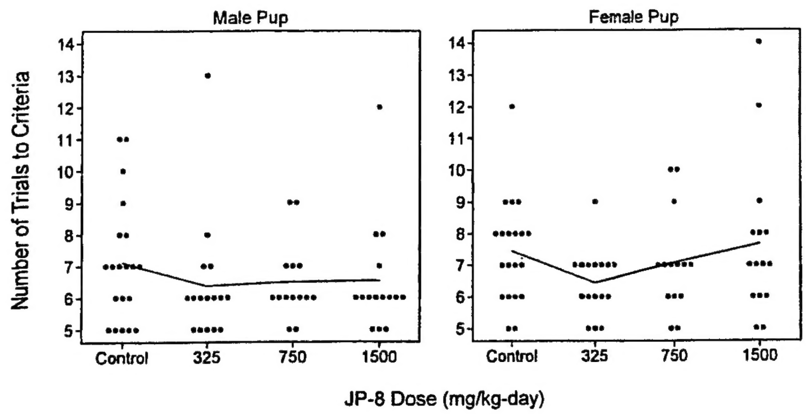

Figure 7. Number of trials to water maze criterion for each pup on PND 70. Line segments connect means from each dose group.

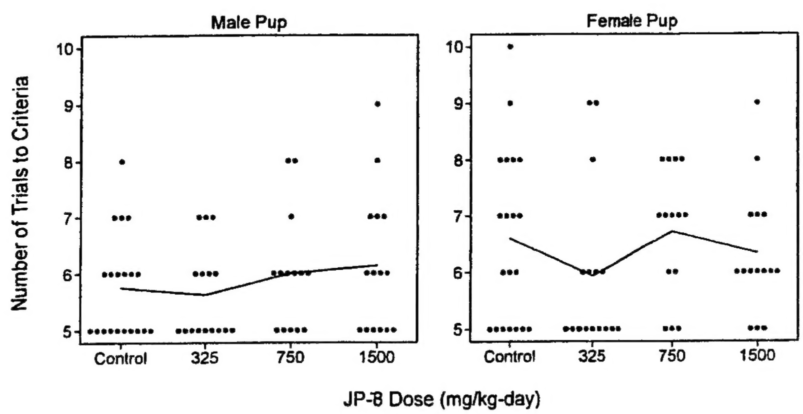

Figure 8. Number of trials to water maze criterion for each pup on PND 77. Line segments connect means from each dose group.

DISCUSSION

Overall, the exposure effects of JP-8 in rat pups were observed only at two different ages and specifically in the task of motor coordination. There was recovery from these effects, as performance on tests administered at later ages showed no treatment effects. Specifically, there were no exposure group differences observed in the simple motor tasks of surface righting and negative geotaxis. However, dose related effects were found in measures of swimming ability where the exposed animals demonstrated poorer performance than animals in the unexposed group on PNDs 8 and 14 only. On both of these days, lower Direction Scores resulted in lower Total Scores in the treated versus control groups. This effect was not lasting since the groups all performed similarly on PNDs 16, 18 and 20, and M-water maze tests on PNDs 70 and 77 revealed no group differences. Thus, the measures suggest a modest effect indicative of possible developmental delays related to brain areas responsible for motor coordination that does not affect motor abilities at later ages. These results warrant further

investigation of motor coordination development, particularly at the ages when treatment differences were observed. The results from the swimming test suggest a problem in general motor coordination, thus it would be appropriate to use a second test of the same abilities to confirm the results. One appropriate task would be a rotorod where the animals are placed on a spinning wheel with variable speed controls and the amount of time the animal can stay on the wheel is measured. While both the swimming task and the rotorod assess motor coordination, the rotorod adds elements of balance and equilibrium. Replicate results in the rotorod test would increase the confidence in the swimming test observations, and more clearly implicate delayed neurodevelopment in the cerebellum, an area of the brain critical for motor coordination and balance.

There are some neurodevelopmental milestones in the cerebellum that correspond to the ages where group differences were observed, and may have influenced the behavioral results. The difference at PND 8 corresponds with the maturation of basket cells in the cerebellum[11]. Also, there are at least two neurodevelopmental endpoints that may be related to the observed group differences at PND 14. First, eye opening occurs approximately at PND 14. Differences in eye opening times, although not assessed in the present study, may have affected the animals' ability to swim forward rather than in a circle, as was suggested by the measure of swimming direction. At this same age, the cerebellum is in the final days of cellular organization and development of functional integrity[11]. Deviations during this final stage may account for coordination deficits seen on PND 14. Comparable motor problems have been reported for rats with known cerebellar damage due to viral infection[12]. The pups with cerebellar injury did not have deficits in righting or geotaxis measures, but there were changes in the more complex motor tests administered on PND 14. The hypothesis of developmental anomalies in the cerebellum, or in other brain areas, could be addressed with morphological analyses. Future JP-8 investigations should include a measure of regional concentrations of hydrocarbons in brains of exposed pups to determine if the cerebellum is a major target area. In addition, morphological data may provide further evidence of delays in neurodevelopment that correspond to the behavioral observations that appear specifically at PNDs 8 and 14.

Although there were no dose-related behavioral effects of JP-8 maternal exposure seen in the negative geotaxis and water maze tests, there were statistically significant differences between male and female pups. For negative geotaxis, female pups required fewer days than male pups to turn their heads upwards. Conversely, in the water maze at PND 77, male pups required fewer trials than female pups to meet the criterion of five consecutive errorless runs. Unrelated to dose group effects, male pups had higher scores than female pups in the swimming development test at PND 8. These effects appear to be related to normal sex differences during development and are not biologically significant for JP-8 exposure.

There are a number of factors that make it difficult to compare the results from this investigation to other studies of JP-8 and neurobehavior. For example, dams in the high dose group in the present study received between 375 to 500 mg JP-8 per day for 21 weeks or 55 to 75 g total. By comparison, in adult inhalation neurobehavioral studies[9, 10], rats were exposed to only 31 to 40 mg per day for 6 weeks or 131 to 175 mg JP-8 total. Therefore, dams in this study received a total of 420 times more JP-8 than adult rats in the inhalation neurobehavioral studies, although daily doses were only about 12 times greater. Also, there are differences associated with the inhalation versus oral routes of administration. The inhalation route may have exposed rats to only the more volatile components of JP-8, while the oral route ensures that all fractions are present in the stomach and available for absorption into the body of the dam. Third, inhalation exposure in adult rats resulted in neurobehavioral effects that were not adequately

assessed in the present study. Specifically, the current investigation did not include any test of central nervous system sensitization. Future studies could include the ARAS, or a more traditional test of sensitization where an animal is administered an amphetamine challenge dose and activity in an open field is assessed. In addition, there was only a single test to assess cognitive abilities in the current investigation, the M-watermaze. This test is not typically used in neurobehavioral assessments, where as the Morris watermaze is a more standard assessment of spatial navigation and cognition. Even so, adult rats in the inhalation study did not demonstrate deficits in simple, or moderately complex tasks with cognitive processing levels similar to that required in the Morris watermaze. The deficiencies were observed only in a complex operant task suggesting follow-up research should include at least one test with greater than moderate complexity in order to detect the effects of JP-8 exposure.

The previously reported NOAEL for these pups for reproductive and developmental endpoints was 750 mg/kg-day, due to decreased pup body weights at the 1500 mg/kg-day maternal dose[8]. Developmental delays were seen at all doses for neurobehavioral endpoints in this report.

CONCLUSIONS

Although developmental delays were not evidenced in all neurobehavioral tasks administered to pups exposed to JP-8 *in utero* and during lactation, a few effects did emerge. The group differences were in tests of motor coordination suggesting a delay in the developing cerebellum of the pups. Recovery from early exposure was evidenced in tests after PND14, as performance in exposed animals did not differ from controls. Thus, the data suggest changes in neurobehavior related to early JP-8 exposure are evident for limited tasks, and this effect is not lasting. A suggested LOAEL would be the lowest dose of 325 mg/kg-day.

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